IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jim Wells et al.	Docket No.:	39750-0002DV1
Serial No.:	09/981,547	Group Art Unit:	1639
Filing Date:	October 17, 2001	Examiner:	Epperson, Jon D.
For:	**METHODS FOR RAPIDLY IDENTIFYING SMALL ORGANIC MOLECULE LIGANDS FOR BINDING TO BIOLOGICAL TARGET MOLECULES**		

DECLARATION OF WARREN L DELANO, PH.D.

Hon. Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

I, Warren L. Delano, Ph.D. declare that:

1. I am a founder and CEO of DeLano Scientific L.L.C., a private software company. Previously, I was Manager of Informatics at Sunesis Pharmaceutical Inc., a company that I help launch in 1998. I am the author of "Unraveling hot spots in binding interfaces: progress and challenges", *Current Opinion in Structural Biology* **12**: 14-20 (2002) that was cited by the Examiner in the Office Communication mailed on July 10, 2003 in connection with the above-identified patent application. My degrees include a Ph.D. in Biophysics from the University of California at San Francisco and a Bachelor or Science in Molecular Biophysics and Biochemistry from Yale University.

2. Having been with Sunesis since its inception, I am familiar with the subject matter of the claimed invention and believe that it is within my area of expertise.

3. I have also read and understand the Office Communication mailed on July 10, 2003 in connection with the above-identified patent application, along with pending claims 58, 59 and 61-66, and a revised claim set which, I understand, is being filed as part of a response to the Office Communication.

4. Having read the Office Communication mailed on July 10, 2003, I disagree with the Examiner's characterization of both the claimed invention and of my publication.

5. The claimed invention is a screening method for identifying a ligand, less than 2000 daltons in size that has the greatest relative affinity for a target protein among a library of compounds. In the method, a target protein is contacted with a library of non-oligomeric organic compounds where each compound is capable of forming a covalent bond with the protein thereby forming a protein-compound conjugate. The ligand having the greatest relative affinity for the target protein among the library of compounds is identified by subjecting the reaction mixture to mass spectrometry analysis and detecting the most abundant target-protein conjugate that is formed.

6. Contrary to the Examiner's assertion, the claimed invention does not require mutations. If a suitable reactive group is already present, then the inventive method can be used on the wild type protein. Illustrative examples include but are not limited to thymidylate synthase to which the Exxaminer referred and cysteine proteases such as caspase-3 (see e.g. Erlanson *et al, Nature Biotechnology* **21**: 308-314 (2003)) that already possess cysteine at the site of interest, which in these cases are the respective active sites.

7. The claimed invention also does not require the identification of "hot spots". In fact, the concept of "hot spots" is moot with respect to the vast majority of potential targets because the sites of interest are already known (e.g., active sites with respect to enzymes and ligand binding sites with respect to receptors). As described in my publication, hot spots are relevant to protein-protein and protein-peptide interactions. Because these interactions involve large surface areas, it was previously believed that small molecule modulators of these types of

interactions may not be possible. The concept of "hot spots" was developed in part by Dr. James Wells (one of the founders of Sunesis Pharmaceuticals, Inc. and a co-inventor of the claimed invention) when he discovered that a surprisingly few residues were responsible for most of the binding interaction. As a result, a hot spot residue is defined as one that when mutated to alanine, gives rise to a distinct drop in the binding constant. In other words, if a hot spot residue in a protein were mutated to alanine, it results in a destabilizing perturbation at the protein interface such that it disrupts its interaction with its protein partner. Because protein-protein interactions appear to be modulated in large part by these hot spot residues, small molecule modulators directed at such residues could be developed to disrupt such interactions for therapeutic benefit.

8. My publication describes the challenges of identifying these hot spots. While it is true that "there are no general patterns of hydrophobiciy, shape or charge that can be used as a basis for predicting which protein atoms will participate in hot spots", hot spots can be and are identified. For example, Piehler and Schreiber describe an approach for measuring kinetics and affinities based on reflectrometric interference spectroscopy which enables efficient large-scale alanine scans and kinetic data. Another approach is described by Weiss that uses phase display of a combinatorial mutant library to simultaneously measure relative residue importance. In fact, the conclusion of my publication is that "recent methodological advances provide efficient experimental means of probing hot spots and enable immediate applications for hot spots in drug discovery" (last sentence of Abstract).

9. Moreover, the consequences of making mutations of hot spot residues are generally different from those of other residues at protein surfaces. Unlike hot spot residues, mutations of surface accessible residues to amino acids containing reactive groups are not generally de-stabilizing to the protein. Consequently, identifying residues for making mutants for use in the claimed invention is well within the skill of the art.

10. Finally, as predicted by my publication, the claimed invention has been useful for identifying ligands on a variety of protein targets, including those involved in protein-protein

interactions. For example, since the publication of my paper, a small molecule modulator of a protein-protein interaction (IL-2) was developed using an embodiment of the claimed invention (*see* Arkin *et al.*, *PNAS* **100**:1603-1608 (2003). As far as I am aware, this is the first instance of a nanomolar small molecule inhibitor of a protein-protein interaction.

I further declare that all statements made herein of my own knowledge are true; and that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 10/3/2003 By: Warren L. Delano

Warren L. Delano, Ph.D.

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DECLARATION OF GARY W. ASHLEY, Ph.D.

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, GARY W. ASHLEY, Ph.D. declare that:

1. I have over twenty-five years of experience in biology and chemistry in both academic institutions and in biotechnology companies. I am currently a Research Fellow at Kosan Biosciences (Hayward, CA). My previous positions include Director of Chemistry (Kosan Biosciences), Senior Scientist (Parnassus Pharmaceuticals Inc., Belmont, CA) and Assistant Professor in the Department of Chemistry and in the Department of Biochemistry, Molecular Biology & Cell Biology (Northwestern University). My degrees include a Bachelor of Science degree in Chemistry from Massachusetts Institute of Technology (1979) and Ph.D. in Chemistry from the University of California at Berkeley (1984). I also have 23 issued U.S. Patents in the fields of molecular biology, chemistry, and drug discovery.

2. I have read the above-identified patent application, and believe that the subject matter of the claimed invention is in an area within my expertise.

3. I have also read and understand pending claims 58, 59 and 61-66, and a revised claim set which, I understand, is being filed as part of a response to an Office communication mailed on July 10, 2003 in connection with the above-identified patent application.

4. The subject matter of the claimed invention is a screening method for identifying a ligand, less than 2000 daltons in size, that has the greatest relative affinity for a target protein among a library of compounds. In the inventive method, a target protein is contacted with a library of non-oligomeric organic compounds where each compound is capable of forming a covalent bond with the protein thereby forming a protein-compound conjugate. The ligand having the greatest relative affinity for the target protein among the library of compounds is identified by subjecting the reaction mixture to mass spectrometry analysis and detecting the most abundant target-protein conjugate that is formed.

5. Having read the specification of the above-referenced patent application, I believe that the invention as claimed is sufficiently described. The formation of the covalently bonded protein-compound conjugate is described, for example, on page 4, lines 7-22 and on page 13, lines 9-32 (disulfide bonded protein compound conjugate); identification of the ligand having the greatest relative affinity by detecting the most abundant protein-compound conjugate is described for example on page 15, lines 15-22; and, analysis by mass spectrometry of the protein-compound conjugate is described, for example, on page 21 lines 17-25. Based on this specific disclosure, and other extensive teaching in the specification concerning the chemistry of the ligands screened, the identity of representative target proteins and suitable covalent bonds, and also in view of my expertise, it is my considered scientific opinion that the claimed screening method is generally applicable for screening a variety of small molecule ligands for a variety of target proteins, using a variety of covalent bonds, as described in the specification and as claimed in the above-identified patent application. From this, I conclude that the inventors were in the possession of the invention as claimed at the time the application was filed.

I further declare that all statements made herein of my own knowledge are true; and that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29 Sept 2003 By: Gary W. Ashley

Gary W. Ashley, Ph.D.

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